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Topical treatment of ichthyoses with 13-cis retinoic acid. A clinical and immunohistochemical study

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In a prospective, double blind, bilaterally paired comparative study, a cream containing 13-cis-retinoic acid (13-cis-RA) 0.1% and the cream base only were applied over a period varying between 4 and 10 weeks in 12 ichthyosis patients (1 patient with autosomal dominant ichthyosis vulgaris (ADIV), 7 patients with X-linked recessive ichthyosis (XRI), 2 patients with bullous congenital ichthyosiform erythroderma of Brocq (BCIE), and 2 patients with erythrodermic lamellar ichthyosis (ELI)). A significant unilateral improvement was found for the clinical parameters of scaling and induration. Clinical improvement was observed in all ichthyosis groups, except for ADIV. Continuation of the treatment beyond the first month, caused a further reduction of skin lesions. Side effects due to the study medication were only minimal and easily controlled by adjusting the application frequency. Biopsies for immunohistochemical examination were taken from representative skin lesions from 9 patients, one before and one from each side after treatment. The keratins 4, 13, and 8, were induced by treatment with topical 13-cis-RA. These keratins could not be detected in the biopsies taken before treatment nor in the biopsies derived from the cream-base treated side. Keratin 4 was induced in 6 of the 9 patients. Induction of keratin 13, visualized with mab 1C7 and 2D7, was found in 1 and 3 patients respectively. Keratin 8, visualized with mab LE41 and M20, was induced in respectively 1 and 3 patients. No clear correlation could be established between expression of keratins and clinical efficacy. Cellular proliferation tended to be increased at the 13-cis-RA treated side. No changes were found for the investigated parameters of inflammation.

Oral retinoids have proven to be very effective in the treatment of ichthyosis [1-3]. Because of side effects, caused by systemic long term therapy with retinoids, topical preparations have regained much of their former importance. Common topical treatment modalities are based on keratolysis and hydration of the epidermis. Although some improvement may be achieved, severe ichthyosis subtypes do not improve sufficiently using these topical treatments.
Topical retinoids affect epidermal proliferation and differentiation [4]. Good responses have been reported in patients with lamellar ichthyosis, ichthyosis vulgaris and to a lesser extent in patients with X-linked ichthyosis and epidermolytic hyperkeratosis, treated with topical tretinoin 0.1% cream. Adverse reactions were erythema, pruritus and irritation and occured in 26 of the 30 patients (87%) [5]. Because of these irritative side effects, the use of topical retinoids has been limited [6].
Recently, Steijlen *et al.* treated one patient with autosomal dominant ichthyosis vulgaris, and seven patients with non-erythrodermic lamellar ichthyosis (NELI), for four weeks using a double-blind, bilaterally paired approach with topical 13-cis-retinoic acid (13-cis-RA) 0.1% and cream base only. A good clinical improvement, in favour of the verum treated side, was observed in two patients with NELI. Immunohistochemical exa-

mination revealed induction of the keratin 4 in two NELI patients [7]. It was suggested that the selective modulation of the cytokeratin pattern might provide an immunohistochemical tool to investigate the mode of action of retinoids.
The aim of the present study was an investigation of the clinical efficacy and tolerability of prolonged topical treatment with 13-cis-RA in one patient with autosomal dominant ichthyosis vulgaris (ADIV), seven patients with X-linked recessive ichthyosis (XRI), 2 patients with bullous congenital ichthyosiform erythroderma of Brocq (BCIE) and 2 patients with erythrodermic lamellar ichthyosis (ELI). Furthermore, immunohistochemical assessment was made of parameters of proliferation, keratinization and inflammation, using monoclonal antibodies, to examine whether a selective modulation of the cytokeratin pattern could be established.

Patients and methods

Patients

One patient with autosomal dominant ichthyosis vulgaris (ADIV), seven with X-linked recessive ichthyosis (XRI), two with bullous congenital ichthyosiform erythroderma of Brocq (BCIE) and two patients with erythrodermic lamellar ichthyosis (ELI) were treated with 13-cis-RA after informed consent was obtained. The XRI-patients had a documented steroid sulphatase deficiency. The NELI- and BCIE-patients had characteristic changes on light- and electronmicroscopic examination. Further details regarding ichthyosis subtype, sex, age, duration of treatment, reason for discontinuation and previous treatments are summarized in *Table I*.

Table I. ***Patient details***

Patient	Diagnosis	Sex	Age	Duration of treatment (weeks)	Reason for discontinuation	Previous treatments
1	ADIV	v	26	10	end of study	urea
2	XRI	m	53	6	end of study*	propylaenglycol
3	XRI	m	17	10	end of study	salicylic acid, urea, ac. lacticum, propylaenglycol
4	XRI	m	30	4	clearance	urea
5	XRI	m	32	6	clearance	urea
6	XRI	m	45	10	end of study	salicylic acid, urea, petrolatum
7	XRI	m	17	10	end of study	none
8	XRI	m	62	10	end of study	salicylic acid, petrolatum
9	BCIE	m	25	10	end of study	unknown
10	BCIE	f	39	9	end of study*	etretimate, petrolatum
11	ELI	f	51	8	end of study*	ac. lacticum, urea, petrolatum
12	ELI	f	37	7	holidays	etretimate, urea, ac. lacticum

* Patients used up all the trial medication.

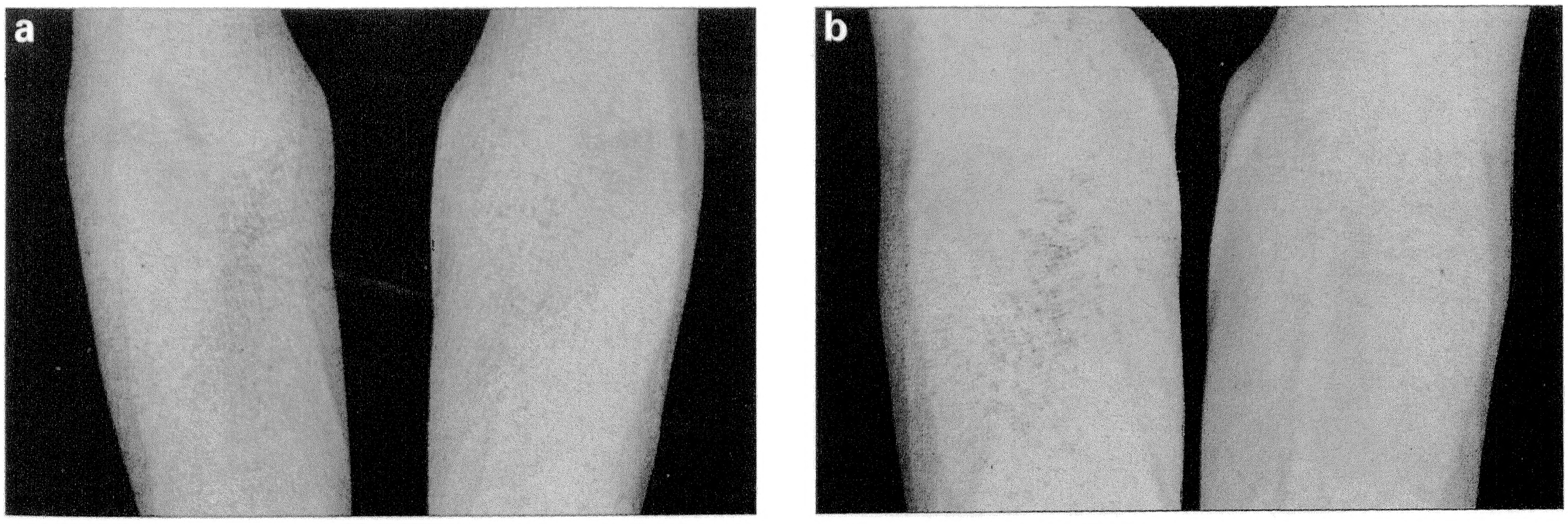

Figure 1. ***(a) XRI-patient with the characteristic phenotype consisting of small brown scales covering the arms with sparing of the flexures, before treatment. (b) After treatment, the 13-cis-RA-treated left side has improved markedly, whereas only slight improvement is observed on the cream base treated right side.***

Topical as well as systemic treatment was stopped respectively 2 and 4 weeks before the trial was started. Using a prospective, bilaterally paired comparative, double-blind approach, 13-cis-RA 0.1% in cream base and cream base only (Hoffmann-La Roche, Basel, Switzerland) were applied twice daily, without occlusion, on a rectangular area of 10 x 10 cm on 2 symmetrical and representative lesions. The creams were applied thinly and evenly during a test period varying between 4 and 10 weeks. In the event of intolerable irritation, patients were instructed to reduce the treatment frequency. In order to establish clinical efficacy, scaling, erythema, and induration were scored using a 4 point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Statistical analysis was carried out using the Wilcoxon signed-rank test for paired data.

Analytical procedures

Biopsies from representative skin lesions within the test areas were taken from 9 patients, one at the start and one from each area at the end of treatment. The keratin antibodies used were the same as used previously [7]. In addition the monoclonal antibody Ki-67 was used to assess proliferation, whereas Leu14, OKT6, WT14, T11, and anti-elastase were used to assess B-lymphocytes, Langerhans cells, macrophages and monocytes, T-lymphocytes, and polymorphonuclear leucocytes respectively. Biopsy procedures and immunohistochemical staining methods were used as described before [7]. An indirect alkalic phosphatase technique was used for staining with the antibodies Ki-67 and anti-elastase.
Ki-67 positive nuclei were counted per millimetre length of section. Presence in the epidermis and dermis of the remaining stained structures were assessed using a semi-quantitative scale:
Epidermis: 0 = no staining; 1 = sporadic staining; 2 = minimal staining; 3 = moderate staining; 4 = moderate-pronounced staining; 5 = pronounced staining; 6 = whole epidermis stained.
Dermis: 0 = absence; 1 = sporadically present; 2 = 1-25% of infiltrate cells stained; 3 = 26-50% of infiltrate cells stained; 4 = 51-75% of infiltrate cells stained; 5 = 76-99% of infiltrate cells stained; 6 = 100% of infiltrate stained.

Results

Clinical response

Patient details are summarized in *Table I. Table II* overviews the clinical efficacy of both verum and cream base.
At the end of the study, 6 of the 12 treated patients showed a half side reduction of scaling in favour of the 13-cis-RA treated side (patient no. 2, 7, 8, 9, 11, 12) *(Figs. 1 and 2)*. In the subgroup with ELI, all patients showed unilateral improvement, whereas in the XRI and BCIE subgroups a half side reduction was achieved in respectively 3 out of 7 and 1 out of 2 patients. No clinical improvement was achieved in the patient with ADIV. Five of these responders also showed improvement of induration (patient 2, 8, 9, 11, 12). The reduction of scaling at the 13-cis-RA side, compared to the cream base side, was statistically significant ($p < 0.05$), as was the reduction of induration ($p < 0.05$). No significant alterations were observed in erythema.
An interindividual variation was observed with regard to duration of treatment needed to achieve a half side reduction. The earliest response was noticed within one week of treatment, while the latest response was not observed before eight weeks of treatment. Within the first month of treatment, a half side reduction was achieved in 5 patients (patient 2, 7, 9, 11, 12). In all of them further improvement was obtained by continuing the treatment beyond the first month.
Itching and irritation of the skin (burning), were reported as side effects in four patients (33%) at the verum treated side only. These side effects though, were only minimal and could be easily controlled by adjusting the application frequency. Irritation did not necessitate discontinuation of the treatment in any patient.

Histological findings

Before treatment RKSE60, recognizing keratin 10, marker for cornifying epithelia, reacted with the suprabasal compartment in a homogeneous pattern. Treatment with topical 13-cis-RA did not change the reaction pattern. The basal cell markers RCK102 and RCK107 stained the basal cell layers in all biopsies taken before and after treatment. No consistent change in staining patterns was observed after treatment with

Table II. ***Clinical effects of topical 13-cis-RA***

Patient	Scaling				Induration			
	Vehicle		13-cis-RA		Vehicle		13-cis-RA	
	Start	End	Start	End	Start	End	Start	End
1	2	2	2	2	2	1	2	1
2	3	3	3	1	3	3	3	1
3	2	1	2	2	1	0	1	0
4	2	0	2	0	1	0	1	0
5	1	0	1	0	1	0	1	0
6	3	1	3	1	2	0	2	0
7	3	3	3	0	1	1	1	1
8	2	1	2	0	1	1	1	0
9	3	2	3	0	3	2	3	0
10	3	1	3	2	3	2	3	2
11	3	3	3	0	2	3	2	0
12	3	3	3	1	3	3	3	1

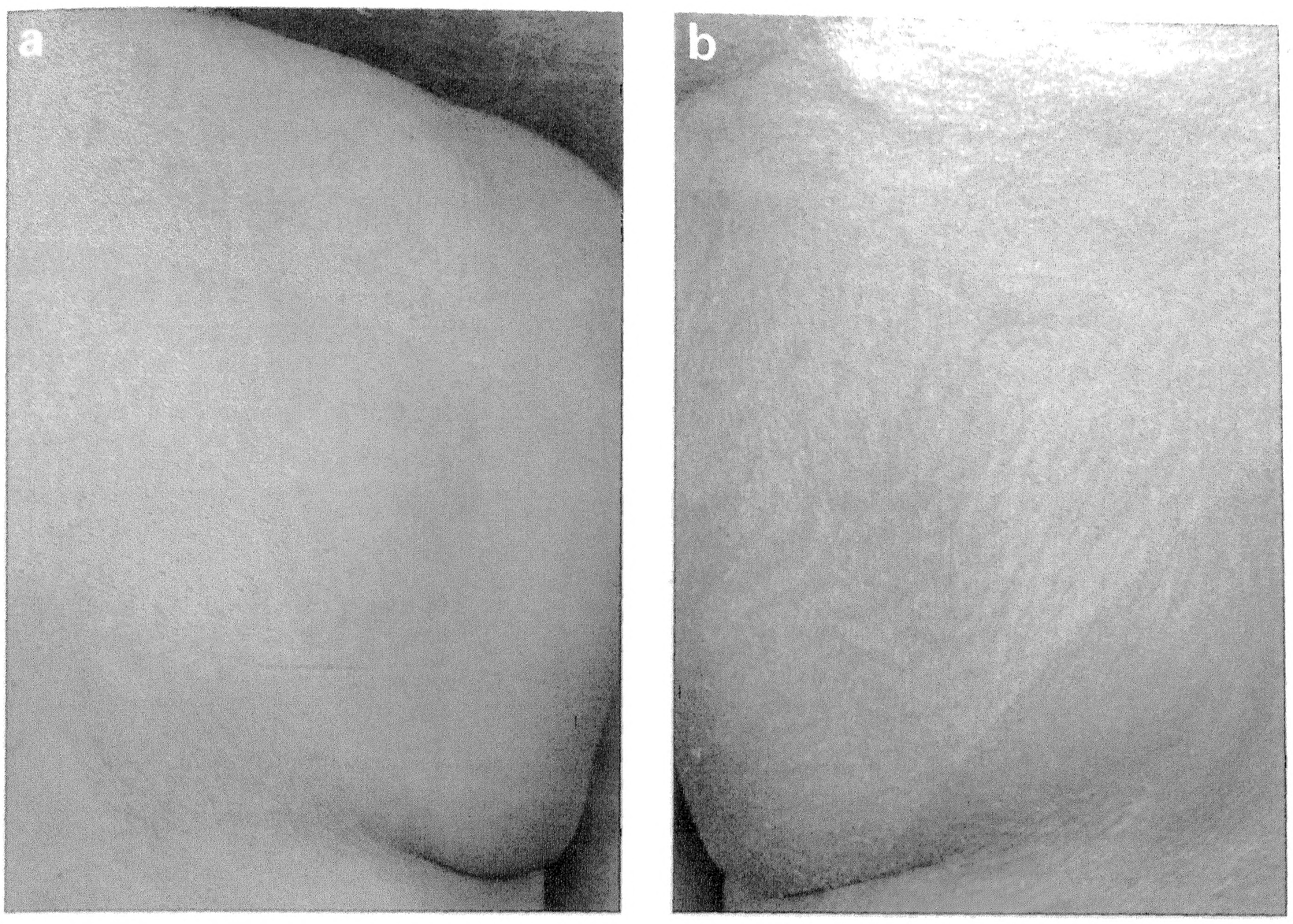

Figure 2. ***(a) After treatment, substantial improvement is seen on the 13-cis-RA treated right side. Scaling is almost completely absent, whereas minimal erythema persists. (b) The scaling on the cream base treated left side, is comparable with the pretreatment state. Erythema is clearly visible, due to absence of reflections in this close-up view.***

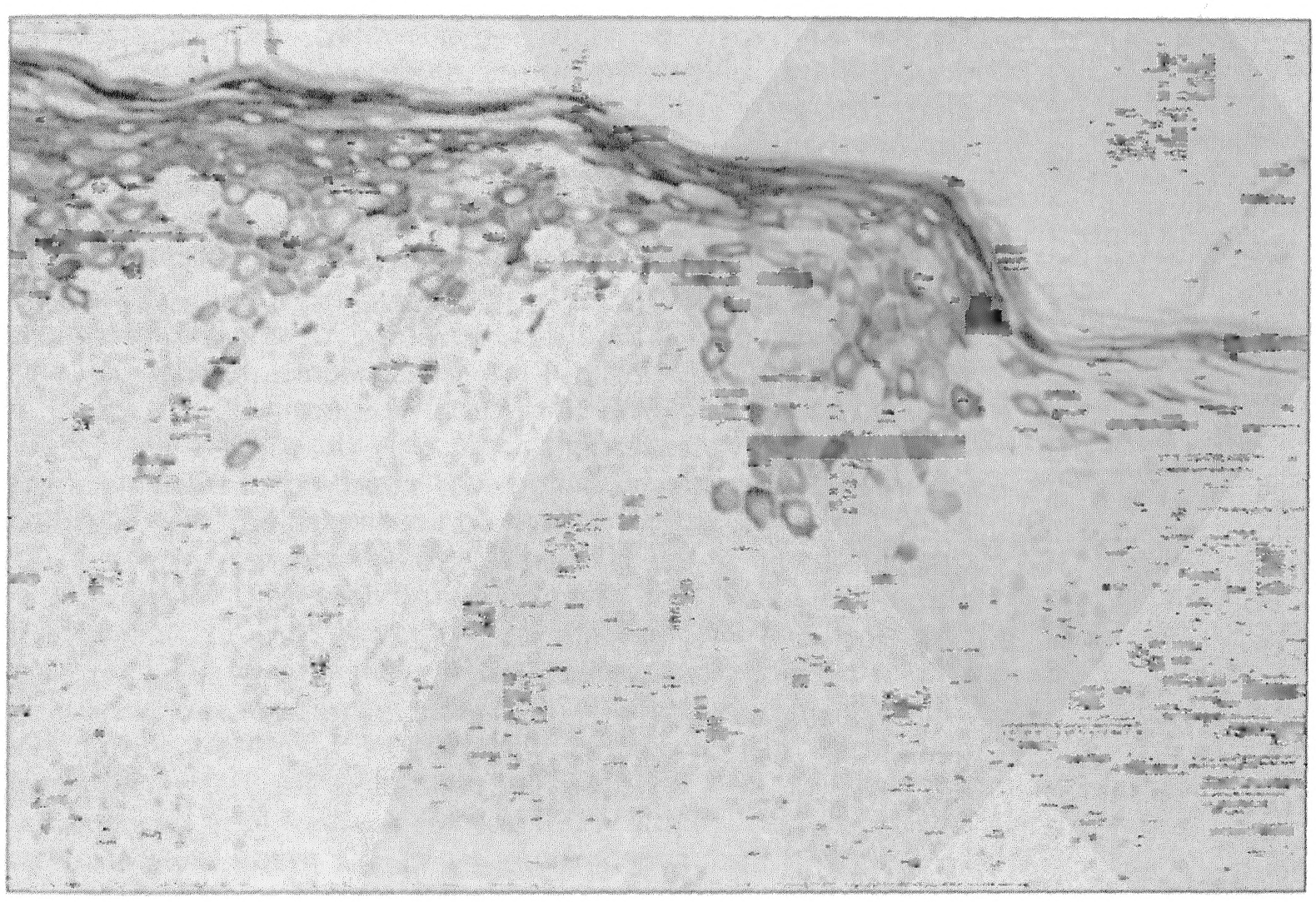

Figure 3. ***Induction of keratin 4 visualized with mab 6B10 in a patient with ELI at the 13-cis-RA treated side. Keratin 4 expression is only found in the upper epidermal cell layers.***

13-cis-RA. The markers for non-cornifying epithelia, *i.e.* antibodies 6B10 to keratin 4, and 1C7 and 2D7 to keratin 13, showed no reaction in the biopsies taken before treatment. After treatment with 13-cis-RA a significant increase of keratin 4 expression (Mab 6B10) was observed ($p < 0.05$). Staining for keratin 4 was absent at baseline, but was present in 6 out of the 9 patients after treatment: staining was moderate in 1 BCIE *(Fig. 3)* and 1 ELI, minimal in 1 ELI, and sporadic in 3 XRI patients *(Table III)*. Staining for keratin 13 was sporadic in 1 BCIE patient, using mab 1C7, and sporadic in 1 ADIV, 1 BCIE, and 1 ELI patient, using mab 2D7. Induction of keratin 4 and 13, was only found at the 13-cis-RA treated side. The markers for simple epithelial cells, *i.e.* antibodies to keratin 7, 18, and 19, showed no epidermal staining in the biopsies taken either before or after treatment. Staining for keratin 8, using the Mab CAM5.2, M20, and LE41, was absent at baseline. After treatment, minimal staining of Mab M20 was found in 1 ADIV, 1 XRI, and 1 ELI patient *(Fig. 4)*, whereas Mab LE41 stained sporadic in 1 ELI patient *(Fig. 5)*, at the 13-cis-RA treated side only. No staining was found with CAM5.2. For Mab KS8.12, staining keratin 13 and proliferation-associated keratin 16, no consistent change in staining pattern was observed after treatment with 13-cis-RA. The average number of cycling cells per millimetre epidermis was 103 before treatment. After treatment, this number was 120 at the 13-cis-RA treated side, and 90 at the cream-base treated side. No changes were found for the investigated parameters of inflammation.

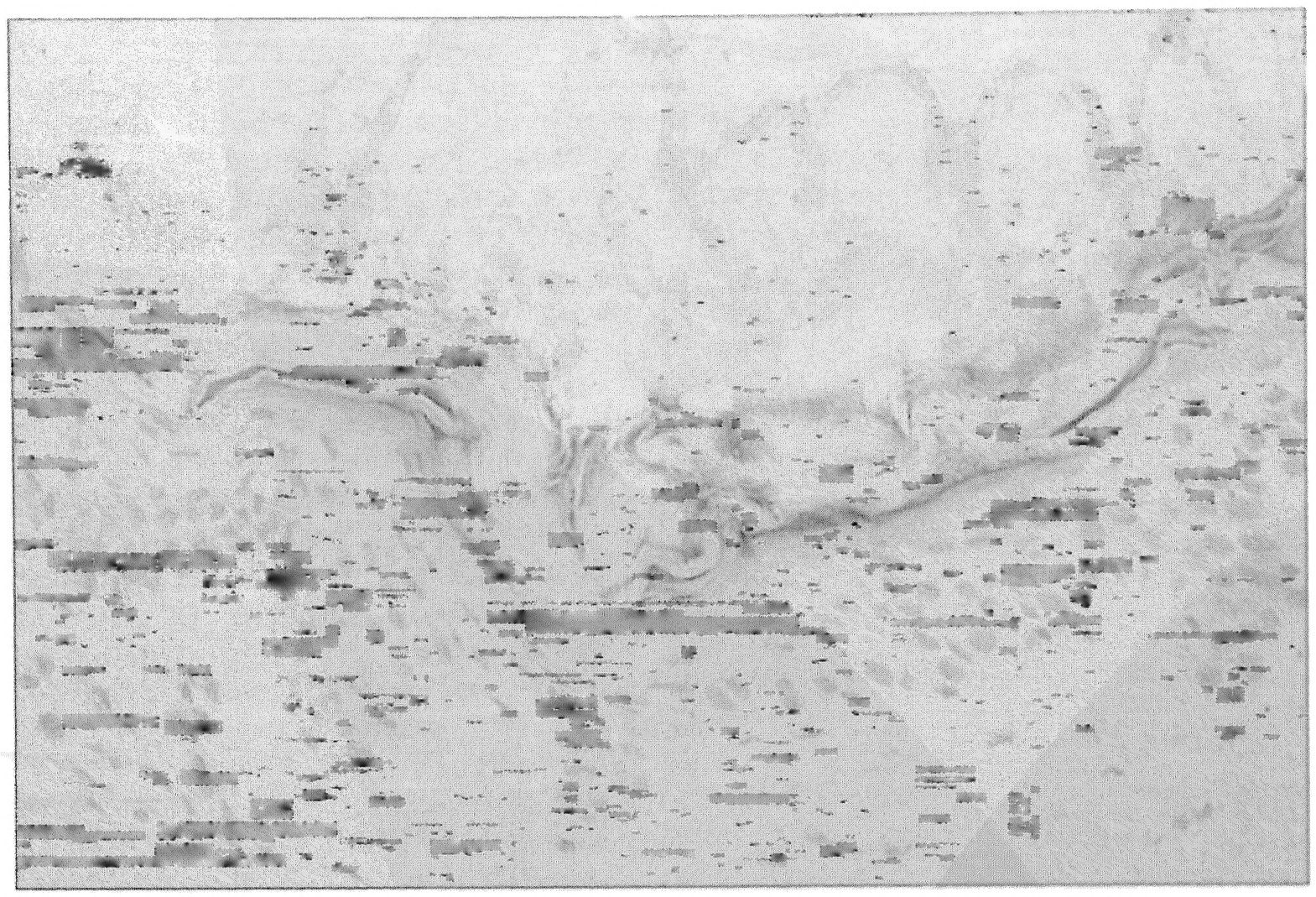

Figure 4. ***Minimal staining with mab M20, indicating keratin 8, in the ADIV patient at the 13-cis-RA treated side. Induction of keratin 8 is confined to the upper granular cell and cornified cell layers.***

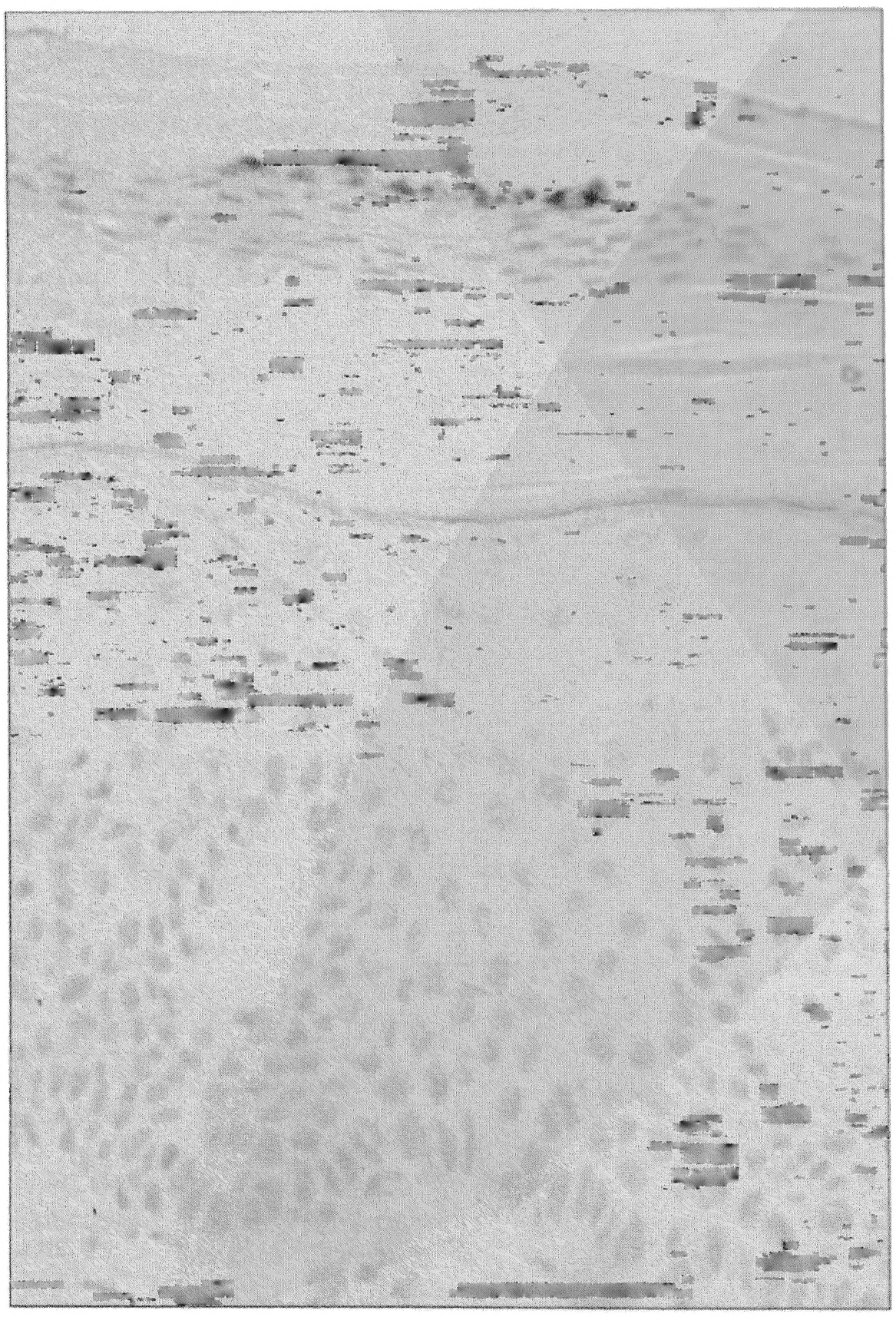

Figure 5. ***Same patient as* Figure 4, *showing expression of mab LE41, staining keratin 8. Induction of keratin 8 is restricted to the upper parakeratotic layers of the stratum corneum.***

Discussion

Twelve ichthyosis patients were treated in a prospective, double-blind, bilaterally paired study, with a cream containing 13-cis-RA 0.1% and its cream-base only, for a period varying between 4 and 10 weeks. A statistically significant improvement of scaling and induration was observed. Clinical improvement was observed in all ichthyosis groups, except for ADIV. Irritation was minimal and could be controlled easily by adjustment of the application frequency. Immunohistochemical assessment of biopsies, revealed a statistically significant induction of keratin 4 in 6/9 patients. Keratin 13 was induced in 3/9 patients. Induction of keratin 8 was found in 3/9 patients. These keratins were only detected at the 13-cis-RA treated side. No induction was observed at baseline. Mab Ki-67, taken as a parameter for proliferation, was elevated after treatment at the 13-cis-RA treated side.

The good results with respect to efficacy and tolerability of topical 13-cis-RA in ichthyosis patients, are in accordance with a previous case report on 2 patients with NELI [7], and demonstrates that other ichthyosis subgroups such as XRI, BCIE, and ELI, may also improve on 13-cis-RA. Additional improvement was observed by extending the treatment period beyond the first month. Further improvement may be achieved by adapting the concentration [8, 9], the mode of application [10] and the cream basis [11]. It is attractive to hypothesize that other retinoids, such as the arotinoids, may be even more effective as a topical treatment for ichthyosis [12].

Keratin 4 and 13, are keratins absent in normal adult skin. Induction has been described after treatment with topical retinoids [7, 13, 14]. This selective modulation of the keratin pattern has been proposed as an immunohistochemical tool to investigate the mode of action of retinoids [7]. Our immunohistochemical results are in conjunction with these previous observations. Furthermore, we found selective induction of keratin 8 at the 13-cis-RA treated skin, which to our knowledge, has not been described previously. In adult skin, keratin 8 is normally expressed in apocrine and eccrine sweat glands [15]. In fetal skin it is expressed in the basal cell layer [16]. It is possible that keratin 8 represents another keratin, selectively modulated by retinoids. As these keratins are normally not expressed during epidermal differentiation,

Table III. ***Immunohistochemical effects of topical 13-cis-RA***

Patient	Keratins and monoclonal antibodies				
	Keratin 4	Keratin 8		Keratin 13	
	6 B 10	M 20	LE 41	1 C 7	2 D 7
1	0	2	0	0	1
2	1	0	0	0	0
3	not done	not done	not done	not done	not done
4	not done	not done	not done	not done	not done
5	1	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	1	2	0	0	0
9	3	0	0	1	1
10	not done	not done	not done	not done	not done
11	2	0	0	0	0
12	3	2	1	0	1

one could argue about the relevance of their induction. No clear correlation could be established between the extent of expression and the clinical efficacy to 13-cis-RA.

In conclusion, topical treatment of ichthyoses with 13-cis-RA 0.1% was followed by a clinical improvement in XRI, BCIE and ELI. Irritation of the skin was minimal and could be controlled easily. In addition to keratin 4 and 13, keratin 8 might represent another keratin induced by retinoids, and thus provide a new immunohistochemical tool to investigate the mode of action of retinoids. The clinical relevance of this keratin expression remains to be established. ■

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